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ASCORBIC ACID, BIOLOGICAL FUNCTION AND CHEMISTRY.

ANNUAL PROGRESS REPORT.

Project Director:
Bert M. Tolbert, Ph.D.

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INTRODUCTION

This is a report on the concluding year of a study in depth on the chemistry and metabolism of ascorbic acid and isoascorbic acid or erythorbic acid. The broad objectives of the research were to study in detail the biochemistry of these compounds to provide the fundamental background for further studies on the nutritive and stress requirement for the soldier for vitamin C. A further aspect of the study was to determine the metabolic interaction of ascorbic acid and erythorbic acid, since the latter is an optical isomer of vitamin C and also a common food additive, especially in military emergency rations.

During the period of this report, three major projects were studied - the first was the nature and enzymic properties of ascorbate sulfatase; the second was whether C-6 oxidation of ascorbic acid was a significant process in ascorbic acid metabolisms; and the third was an effort to prepare a C-6 oxidized ascorbic acid by synthetic methods, so that its presence or absence in biological tissue could be tested.

REPORT ON ASCORBATE SULFATE SULFOHYDROLASE

Ascorbate is a ubiquitous metabolite of ascorbic acid in higher animals. It is a vitamin in several species of fish. But most important of all, it is stable to air oxidation and does not hydrolyze at neutral pH's. Thus it has special nutritive value for fish. Man surely ingests a considerable amount in his diet.

If ascorbate sulfate is to serve as a source of ascorbic acid there should be an enzyme that can hydrolyze this compound back to ascorbic acid. We have discovered such a compound in a number of animals, and examined in detail the properties of the enzyme from cow liver. This report is attached.

Two interesting questions remain: ascorbate sulfatase is very similar to an important animal enzyme called aryl sulfatase A. The absence of aryl sulfatase A in humans results in the genetic disease metachromatic leukodystrophy. What is the relationship between these enzymes and what is their metabolic role? The other problem is whether ascorbate sulfatase serves to hydrolyze any significant amount of ascorbate sulfate, and whether the ascorbate sulfate has a biological role. At present, it seems ascorbate is both an excretion form of ascorbic acid, and has biological function, perhaps as an hypolipodemic agent.

REPORT ON C-6 OXIDATION OF ASCORBIC ACID

Whether there is significant C-6 oxidation of ascorbic acid in higher animals was tested in two ways. First, a periodate degradation for ascorbic acid was developed, see attached reprint (1976) and this method was applied to urine of monkeys and rats given [6-^{14}C] - ascorbic acid. The experiments, see attached reprint, showed that about 45% of all ascorbic acid metabolites were no longer in the $-CH_2OH$ oxidation state, characteristic of ascorbic acid.

To confirm this result [6-^{3}H] - ascorbic acid was injected into monkeys and their excretion of ^{3}H in urine measured. The excretion was determined for ^{3}H-water in the urine and for organic bound ^{3}H in urine. This study, see attached reprint, showed that again about 45% of the tritum had been released from the ascorbic acid metabolites and appeared in the urine.

The biological significance of this side chain metabolism of ascorbic acid could be related to some special function of ascorbic acid - vitamin C.

PREPARATION OF SACCHAROASCORBIC ACID

If ascorbic acid is subject to C-6 oxidation, a likely product is ascorbic acid with a side chain terminal carbon oxidized to a carboxyl group, or saccharoascorbic acid. Preparation of this compound was attempted in various ways. Success was finally achieved using the method shown in Figure 1. Details of the procedure were published in 1978.

Harkrader, R.J., Plunkett, L.M., and Tolbert, B.M., "Periodate Degradation of Labeled Ascorbic Acid," Analytical Biochemistry 72, 310-314 (1976).

About a month after the new contract year began, October 15, 1975, support for this work was terminated effective January 15th. This date was later extended to June 30th with no additional funds to allow a more orderly termination of the graduate students who were doing the work described in this report.

Their work has been of outstanding quality and their results have been the basis of significant further studies on the metabolism of ascorbic acid. The problem remains to obtain solid scientific results from which optimum intakes of ascorbic acid can be determined for the soldier under various conditions to maximize his ability as a military man.

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